Postprint: Study on Chemical Constituents of Ipomoea purpurea

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Abstract

Ipomoea purpurea is a plant with abundant resources, but studies on the whole herb are scarce; therefore, systematic investigation of its chemical constituents and biological activities is necessary. In this study, the chemical constituents of the dried whole herb of Ipomoea purpurea collected from Dali, Yunnan, were systematically investigated. Extraction was performed by cold maceration with 75% ethanol, and the resulting extract was dispersed in water and successively partitioned with ethyl acetate and n-butanol. Using column chromatography on various stationary phases including silica gel, Sephadex LH-20, and RP-18, combined with recrystallization, twelve monomeric compounds were isolated from the ethyl acetate fraction of the ethanol extract. These compounds were identified by modern spectroscopic techniques as friedelin (1), -friedelinol (2), -amyrin (3), -amyrin (4), 6 -hydroxystigmast-4-en-3-one (5), daucosterol (6), -sitosterol (7), stigmasterol (8), 7-hydroxycoumarin (9), p-coumaric acid-p-hydroxyphenethyl ester (10), kaempferol coumaroyl glucopyranoside (11), and glyceryl monopalmitate (12). Compounds 2–5 and 10–12 were isolated from this genus for the first time.

Full Text

Chemical Constituents of Pharbitis purpurea

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Abstract: Pharbitis purpurea is a plant with abundant resources, yet systematic studies on its whole herb remain scarce. Investigating its chemical constituents and biological activities is therefore essential. This study presents a systematic investigation of the chemical constituents from the dried whole

herb of P. purpurea collected in Dali, Yunnan. The plant material was extracted with 75% ethanol using cold maceration, and the resulting extract was concentrated and suspended in water before successive partitioning with ethyl acetate and n-butanol. From the ethyl acetate fraction of the ethanol extract, twelve compounds were isolated using various chromatographic methods including silica gel, Sephadex LH-20, and RP-18 column chromatography, as well as recrystallization. Modern spectroscopic techniques identified these compounds as friedelin (1), -friedelinol (2), -amyrin (3), -amyrin (4), 6 -hydroxystigmast-4-en-3-one (5), daucosterol (6), -sitosterol (7), stigmasterol (8), umbelliferone (9), p-hydroxyphenylethanol p-coumarate (10), kaempferol-3- -D-(6-O-cis-p-coumaroyl)glucopyranoside (11), and glyceroylmonopalmitate (12). Compounds 2–5 and 10–12 were isolated from the genus Pharbitis for the first time.

Keywords: Pharbitis purpurea, chemical constituents, triterpene, steroid
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Introduction

Plants of the genus Pharbitis (or Ipomoea, Convolvulaceae) are annual or perennial twining herbs comprising approximately 24 species worldwide, primarily distributed in temperate and subtropical regions. Three species occur in China, found across most of the country. Modern research indicates that Pharbitis species are rich in chemical constituents, mainly including flavonoids, terpenoids, phenylpropanoids, and volatile oils. Some isolated compounds from these plants have demonstrated cytotoxic, antitumor, and insecticidal activities.

Pharbitis purpurea (L.) Voigt (also known as Ipomoea purpurea), native to tropical America, has become naturalized across most of China, growing in fields, roadsides, residential areas, and forest valleys from flatlands up to 2,800 m elevation, either cultivated or wild. While the seeds of this plant have been studied extensively as a traditional Chinese medicinal material (one of the sources of "Pharbitidis Semen"), the whole herb has not been investigated systematically. Our preliminary large-scale screening of medicinal plants revealed that the whole herb extract of P. purpurea possesses notable analgesic activity, underscoring the necessity for systematic investigation of its chemical constituents and bio-

logical activities.

To understand the fundamental chemical profile of this plant and establish a foundation for subsequent bioactivity studies and development, we conducted a systematic isolation and identification of chemical constituents from the whole herb of P. purpurea. Using 75% ethanol as the extraction solvent, we employed silica gel, Sephadex LH-20, RP-18, and other chromatographic materials combined with recrystallization to isolate twelve compounds from the ethyl acetate fraction of the ethanol extract. Structural identification was performed using ^{1}H- and ^{13}C-NMR spectroscopy, revealing that the isolated constituents are primarily triterpenoids and steroids.

Materials and Methods

1.1 Instruments and Materials NMR spectra were recorded on a Bruker Avance III-400 spectrometer (Bruker, Germany) with TMS as internal standard. Column chromatography silica gel and TLC silica gel plates GF254 were purchased from Qingdao Marine Chemical Factory. A RE-52AA rotary evaporator (Shanghai Yarong Biochemical Instrument Factory) and an AL204 electronic balance (Mettler-Toledo Shanghai Co., Ltd.) were used. Sephadex LH-20 was obtained from Amersham Biosciences (Sweden), D101 macroporous resin from Tianjin Bohong Resin Technology Co., Ltd., RP-18 (40–75 m) from Fuji (Japan), and MCI CHP-20 (75–150 m) from Mitsubishi (Japan). Petroleum ether, chloroform, ethyl acetate, acetone, and methanol were industrial-grade solvents redistilled before use, while n-butanol and isopropanol were analytical pure (Shanghai Chemical Co., Ltd.). Compounds were visualized by spraying with 10% H SO followed by heating, or by iodine vapor staining.

Plant material was collected in July 2017 from Jiangwei, Dali, Yunnan, and identified as Pharbitis purpurea (L.) Voigt by Dr. Zhang Dequan of the College of Pharmacy and Chemistry, Dali University. A voucher specimen (No. 20170709-2) is deposited in the research group of Professor Jiang Bei at the Institute of Materia Medica, Dali University.

1.2 Extraction and Isolation Dried whole herb of P. purpurea (4 kg) was powdered and extracted three times with 75% ethanol by cold maceration. The combined extracts were concentrated to yield 749 g of crude extract, which was suspended in water and successively partitioned with ethyl acetate and n-butanol. The ethyl acetate fraction (117 g) was mixed with silica gel (80–100 mesh) and subjected to silica gel column chromatography (300–400 mesh) using a chloroform-acetone gradient (1:0 to 0:1) as eluent. Based on TLC analysis, ten fractions (A–J) were combined.

Fraction B was further separated by silica gel column chromatography using a petroleum ether-ethyl acetate gradient (1:0 to 0:1). Subfraction B2 was recrystallized from petroleum ether-ethyl acetate to yield compound **1** (50 mg).

Subfraction B3 was recrystallized from petroleum ether to give compound **2** (39 mg). Subfractions B4–5 were recrystallized from petroleum ether to afford compounds **3** (22 mg) and **4** (42 mg).

Fraction D was subjected to silica gel column chromatography (petroleum ether-acetone 1:0 to 0:1). Subfraction D2 was washed repeatedly with methanol to obtain compounds **7** (106 mg) and **8** (69 mg).

Fraction E was purified by MCI column chromatography (60%–100% methanol/water). Subfraction E2 was separated by silica gel column chromatography (chloroform-acetone 1:0 to 0:1). E2-1 was washed with acetone to yield compound **9** (15 mg), while E2-6 was washed with methanol to give compound **10** (15 mg). Subfraction E4 was washed with methanol to afford compound **12** (66 mg). Subfraction E7 was further separated by silica gel column chromatography (chloroform-isopropanol 1:0 to 0:1), RP-18 column chromatography (50%–100% methanol/water), and Sephadex LH-20 gel column chromatography (chloroform-methanol 1:1) to obtain compound **5** (6 mg).

The methanol-insoluble portion of fraction I was washed with chloroform-methanol to yield compound **6** (27.9 mg). The remaining sample was purified by MCI column chromatography (50%–100% methanol/water) and silica gel column chromatography (chloroform-methanol 1:0 to 0:1) to obtain compound **11** (779 mg).

[Figure 1: see original paper] Chemical structures of compounds 1–12

Structural Identification

Compound 1 was obtained as colorless needles (petroleum ether-ethyl acetate) with the molecular formula C H O. ^{1}H-NMR (400 MHz, CDCl) : 1.17 (3H, s, Me-28), 1.04 (3H, s, Me-27), 1.00 (3H, s, Me-29), 0.99 (3H, s, Me-26), 0.94 (3H, s, Me-30), 0.87 (3H, d, J = 6.5 Hz, Me-23), 0.86 (3H, s, Me-25), 0.71 (3H, s, Me-24). ^{13}C-NMR (100 MHz, CDCl) : 22.3 (t, C-1), 41.5 (t, C-2), 213.3 (s, C=O), 58.2 (d, C-4), 42.2 (s, C-5), 41.3 (t, C-6), 18.2 (t, C-7), 53.1 (d, C-8), 37.4 (d, C-9), 59.5 (d, C-10), 35.6 (t, C-11), 30.5 (t, C-12), 39.7 (s, C-13), 38.3 (s, C-14), 32.4 (t, C-15), 36.0 (t, C-16), 30.0 (s, C-17), 42.8 (d, C-18), 35.3 (t, C-19), 28.2 (s, C-20), 32.8 (t, C-21), 39.3 (t, C-22), 6.8 (q, C-23), 14.7 (q, C-24), 18.0 (q, C-25), 20.3 (q, C-26), 18.7 (q, C-27), 32.1 (q, C-28), 31.8 (q, C-29), 35.0 (q, C-30). These data are consistent with literature values (Klass et al., 1992), identifying compound **1** as friedelin.

Compound 2 (C H O) was obtained as a white amorphous powder. ^{1}H-NMR (400 MHz, C D N) : 3.99 (1H, brs, H-3), 1.32 (3H, s, Me-24), 1.25 (3H, s, Me-28), 1.20 (3H, d, J = 6.5 Hz, Me-23), 1.11 (3H, s, Me-30), 1.10 (3H, s, Me-27), 1.05 (3H, s, Me-29), 1.05 (3H, s, Me-26), 0.99 (3H, s, Me-27). ^{13}C-NMR (100 MHz, C D N) : 17.1 (t, C-1), 36.9 (t, C-2), 71.9 (d, C-3), 50.5 (d, C-4), 39.1 (s, C-5), 42.8 (t, C-6), 18.5 (t, C-7), 54.0 (d, C-8), 38.0 (d, C-9), 62.5 (d, C-10),

36.5 (t, C-11), 31.4 (t, C-12), 39.0 (s, C-13), 40.4 (s, C-14), 33.1 (t, C-15), 39.9 (t, C-16), 30.7 (s, C-17), 43.7 (d, C-18), 33.7 (t, C-19), 28.8 (s, C-20), 36.1 (t, C-21), 37.0 (t, C-22), 12.9 (q, C-23), 17.4 (q, C-24), 19.0 (q, C-25), 20.7 (q, C-26), 19.3 (q, C-27), 32.7 (q, C-28), 35.5 (q, C-29), 32.4 (q, C-30). These data are consistent with literature values (Duwiejua et al., 1999), identifying compound **2** as -friedelinol.

Compound 3 (C H O) was obtained as colorless needles (petroleum ether). ^{1}H-NMR (400 MHz, CDCl) : 5.18 (1H, t, J = 3.7 Hz, H-12), 3.23 (1H, dd, J = 10.8, 4.4 Hz, H-3a), 1.06 (3H, s, Me-27), 0.96 (3H, s, Me-26), 0.95 (3H, s, Me-25), 0.91 (3H, s, Me-30), 0.87 (3H, s, Me-23), 0.80 (3H, s, Me-28), 0.79 (3H, s, Me-24), 0.79 (3H, s, Me-29). ^{13}C-NMR (100 MHz, CDCl) : 38.6 (t, C-1), 27.2 (t, C-2), 79.0 (d, C-3), 38.8 (s, C-4), 55.2 (d, C-5), 18.4 (t, C-6), 32.6 (t, C-7), 38.8 (s, C-8), 47.6 (d, C-9), 36.9 (s, C-10), 23.5 (t, C-11), 121.7 (t, C-12), 145.2 (s, C-13), 41.7 (s, C-14), 26.2 (t, C-15), 26.9 (t, C-16), 32.5 (s, C-17), 47.2 (d, C-18), 46.8 (t, C-19), 31.1 (s, C-20), 34.7 (t, C-21), 37.1 (t, C-22), 28.1 (q, C-23), 15.5 (q, C-24), 15.6 (q, C-25), 16.8 (q, C-26), 26.0 (q, C-27), 28.4 (q, C-28), 33.4 (q, C-29), 23.7 (q, C-30). These data are consistent with literature values (Fukunaga et al., 2009), identifying compound **3** as -amyrin.

Compound 4 (C H O) was obtained as colorless needles (petroleum ether). ^{1}H-NMR (400 MHz, CDCl) : 5.12 (1H, t, J = 3.7 Hz, H-12), 3.21 (1H, dd, J = 10.8, 4.4 Hz, H-3), 1.13 (3H, s, Me-27), 0.99 (3H, s, Me-23), 0.99 (3H, s, Me-26), 0.93 (3H, s, Me-24), 0.87 (3H, overlap, Me-29), 0.86 (3H, overlap, Me-30), 0.83 (3H, s, Me-28), 0.79 (3H, s, Me-25). ^{13}C-NMR (100 MHz, CDCl) : 38.8 (t, C-1), 27.3 (t, C-2), 79.1 (d, C-3), 38.8 (s, C-4), 55.2 (d, C-5), 18.4 (t, C-6), 32.9 (t, C-7), 40.0 (s, C-8), 47.7 (d, C-9), 36.9 (s, C-10), 23.4 (t, C-11), 124.4 (t, C-12), 139.6 (s, C-13), 42.1 (s, C-14), 26.6 (t, C-15), 28.1 (t, C-16), 33.8 (s, C-17), 59.0 (d, C-18), 39.7 (t, C-19), 39.6 (s, C-20), 31.1 (t, C-21), 41.5 (t, C-22), 28.1 (q, C-23), 15.6 (q, C-24), 15.7 (q, C-25), 16.9 (q, C-26), 23.3 (q, C-27), 28.8 (q, C-28), 17.5 (q, C-29), 21.4 (q, C-30). These data are consistent with literature values (Lee et al., 2003), identifying compound **4** as -amyrin.

Compound 5 (C H O) was obtained as colorless needles. ^{1}H-NMR (400 MHz, CDCl) : 5.81 (1H, s, H-4), 4.34 (1H, brs, H-6), 1.37 (3H, s, Me-19), 0.92 (3H, d, J = 6.3 Hz, Me-21), 0.84, 0.83, 0.80 (each 3H, overlap, Me-26, 27, 29), 0.74 (3H, s, Me-18). ^{13}C-NMR (100 MHz, CDCl) : 37.2 (t, C-1), 34.4 (t, C-2), 200.7 (s, C-3), 126.4 (d, C-4), 168.7 (s, C-5), 73.4 (d, C-6), 38.7 (t, C-7), 29.8 (d, C-8), 53.7 (d, C-9), 38.1 (s, C-10), 21.1 (t, C-11), 39.7 (t, C-12), 42.6 (s, C-13), 56.0 (d, C-14), 24.3 (t, C-15), 28.3 (t, C-16), 56.2 (d, C-17), 12.1 (q, C-18), 19.7 (q, C-19), 36.3 (d, C-20), 19.2 (q, C-21), 34.0 (t, C-22), 26.2 (t, C-23), 45.9 (d, C-24), 29.2 (d, C-25), 20.0 (q, C-26), 18.9 (q, C-27), 23.2 (t, C-28), 12.2 (q, C-29). These data are consistent with literature values (Niu et al., 2001), identifying compound **5** as 6 -hydroxystigmast-4-en-3-one.

Compound 6 (C H O) was obtained as a white amorphous powder. ^{1}H-NMR (400 MHz, C D N) : 5.36 (1H, brs, H-6), 5.10 (1H, d, J = 7.3 Hz, H-1'), 4.56 (1H, dd, J = 11.8, 2.4 Hz, H-6 a), 4.45 (1H, dd, J = 11.8, 5.2 Hz, H-6 b), 4.32

(2H, overlap, H-4 , 5), 4.10 (1H, m, H-3), 4.01 (2H, overlap, H-2 , 3), 1.00 (3H, d, J = 6.4 Hz, Me-21), 0.95 (3H, s, Me-19), 0.94 (3H, overlap, Me-26), 0.90 (3H, overlap, Me-27), 0.89 (3H, overlap, Me-29), 0.67 (3H, s, Me-18). ^{13}C-NMR (100 MHz, C D N) : 37.6 (t, C-1), 30.3 (t, C-2), 78.7 (d, C-3), 40.5 (t, C-4), 141.0 (s, C-5), 122.0 (d, C-6), 34.3 (t, C-7), 32.3 (d, C-8), 50.4 (d, C-9), 37.6 (s, C-10), 21.4 (t, C-11), 39.4 (t, C-12), 42.6 (s, C-13), 57.0 (d, C-14), 24.6 (t, C-15), 28.7 (t, C-16), 56.3 (d, C-17), 12.3 (q, C-18), 19.5 (q, C-19), 36.5 (d, C-20), 19.3 (q, C-21), 34.3 (t, C-22), 26.5 (t, C-23), 46.1 (d, C-24), 29.5 (d, C-25), 20.1 (q, C-26), 19.5 (q, C-27), 32.3 (t, C-28), 12.3 (q, C-29), 102.7 (d, C-1'), 75.4 (d, C-2'), 78.2 (d, C-3'), 71.8 (d, C-4'), 78.7 (d, C-5'), 63.0 (t, C-6'). These data are consistent with literature values (Cho et al., 1987), identifying compound **6** as daucosterol.

Compound 7 (C H O) was obtained as colorless needles (petroleum ether-ethyl acetate). ^{1}H-NMR (400 MHz, CDCl) : 5.35 (1H, brs, H-6), 3.52 (1H, m, H-3), 1.01 (3H, s, Me-19), 0.92 (3H, d, J = 6.5 Hz, Me-21), 0.85, 0.82, 0.81 (each 3H, overlap, Me-26, 27, 29), 0.68 (3H, s, Me-18). ^{13}C-NMR (100 MHz, CDCl) : 37.3 (t, C-1), 31.6 (t, C-2), 71.8 (d, C-3), 42.3 (t, C-4), 140.8 (s, C-5), 121.7 (d, C-6), 31.9 (t, C-7), 31.6 (d, C-8), 50.1 (d, C-9), 37.3 (s, C-10), 21.9 (t, C-11), 39.8 (t, C-12), 42.3 (s, C-13), 56.8 (d, C-14), 24.3 (t, C-15), 29.1 (t, C-16), 56.1 (d, C-17), 12.0 (q, C-18), 19.4 (q, C-19), 36.5 (d, C-20), 19.4 (q, C-21), 33.9 (t, C-22), 26.1 (t, C-23), 45.8 (d, C-24), 29.2 (d, C-25), 19.8 (q, C-26), 18.8 (q, C-27), 24.3 (t, C-28), 11.9 (q, C-29). These data are consistent with literature values (Nirmal et al., 2012), identifying compound **7** as -sitosterol.

Compound 8 (C H O) was obtained as colorless needles (petroleum ether-ethyl acetate). ^{1}H-NMR (400 MHz, CDCl) : 5.35 (1H, brs, H-6), 5.15 (1H, dd, J = 15.2, 8.5 Hz, H-23), 5.01 (1H, dd, J = 15.2, 8.5 Hz, H-22), 3.52 (1H, m, H-3), 1.01 (3H, s, Me-19), 0.92 (3H, d, J = 6.5 Hz, Me-21), 0.84, 0.83, 0.80 (each 3H, overlap, Me-26, 27, 29), 0.70 (3H, s, Me-18). ^{13}C-NMR (100 MHz, CDCl) : 37.4 (t, C-1), 31.5 (t, C-2), 71.8 (d, C-3), 42.6 (t, C-4), 140.8 (s, C-5), 121.7 (d, C-6), 32.4 (t, C-7), 32.1 (d, C-8), 50.7 (d, C-9), 36.5 (s, C-10), 21.2 (t, C-11), 40.5 (t, C-12), 42.2 (s, C-13), 56.2 (d, C-14), 23.1 (t, C-15), 29.2 (t, C-16), 56.9 (d, C-17), 12.1 (q, C-18), 19.8 (q, C-19), 40.5 (d, C-20), 21.2 (q, C-21), 138.3 (d, C-22), 129.3 (d, C-23), 51.2 (d, C-24), 31.9 (d, C-25), 19.0 (q, C-26), 21.2 (q, C-27), 25.4 (t, C-28), 12.3 (q, C-29). These data are consistent with literature values (Tanaka et al., 2013), identifying compound **8** as stigmasterol.

Compound 9 (C H O) was obtained as a light yellow powder. ^{1}H-NMR (400 MHz, CD OD) : 7.84 (1H, d, J = 9.5 Hz, H-4), 7.44 (1H, d, J = 8.5 Hz, H-5), 6.79 (1H, dd, J = 8.5, 2.3 Hz, H-6), 6.70 (1H, d, J = 2.3 Hz, H-8), 6.18 (1H, d, J = 9.5 Hz, H-3). ^{13}C-NMR (100 MHz, CD OD) : 163.7 (s, C-2), 112.3 (d, C-3), 146.0 (d, C-4), 113.1 (s, C-4a), 130.7 (d, C-5), 114.5 (d, C-6), 163.1 (s, C-7), 103.4 (d, C-8), 157.2 (s, C-8a). These data are consistent with literature values (An et al., 2005), identifying compound **9** as umbelliferone.

Compound 10 (C H O) was obtained as a white powder. ^{1}H-NMR (400 MHz, C D N) : 8.15 (1H, d, J = 15.6 Hz, H-7), 7.56 (2H, d, J = 8.6 Hz, H-2, 6),

7.27 (2H, d, J = 8.5 Hz, H-2 , 6), 7.16 (2H, d, J = 8.4 Hz, H-3 , 5), 7.13 (2H, d, J = 8.6 Hz, H-3, 5), 6.90 (1H, d, J = 15.6 Hz, H-8), 3.89 (2H, m, H-8), 3.03 (2H, t, J = 7.2 Hz, H-7). ^{13}C-NMR (100 MHz, C D N) : 127.3 (s, C-1), 130.3 (d, C-2, 6), 117.0 (d, C-3, 5), 160.8 (s, C-4), 140.5 (d, C-7), 119.7 (d, C-8), 167.2 (s, C-9), 130.8 (d, C-1), 130.7 (d, C-2 , 6), 116.6 (d, C-3 , 5), 157.7 (s, C-4), 36.0 (s, C-7), 42.3 (s, C-8). These data are consistent with literature values (Deng and Zhao, 1993), identifying compound **10** as p-hydroxyphenylethanol p-coumarate.

Compound 11 (C H O) was obtained as a yellow powder. ^{1}H-NMR (400 MHz, CD OD) : 7.96 (2H, d, J = 8.9 Hz, H-2 , 6), 7.37 (1H, d, J = 16.0 Hz, H-3), 7.28 (2H, d, J = 8.5 Hz, H-5 , 9), 6.77 (4H, overlap, H-3 , 5 , H-6 , 8), 6.27 (1H, d, J = 2.0 Hz, H-8), 6.10 (1H, d, J = 2.0 Hz, H-6), 6.04 (1H, d, J = 16.0 Hz, H-2), 5.22 (1H, d, J = 7.5 Hz, H-1), 4.28 (1H, dd, J = 11.8, 2.3 Hz, H-6 a), 4.17 (1H, dd, J = 11.8, 6.6 Hz, H-6 b), 3.45 (3H, overlap, H-2 , 3 , 5), 3.32 (1H, overlap, H-4). ^{13}C-NMR (100 MHz, CD OD) : 159.3 (s, C-2), 135.2 (s, C-3), 179.4 (s, C-4), 162.9 (s, C-5), 100.0 (d, C-6), 166.0 (s, C-7), 94.8 (d, C-8), 158.4 (s, C-9), 105.5 (s, C-10), 122.7 (s, C-1), 132.2 (d, C-2 , 6), 116.0 (d, C-3 , 5), 161.2 (s, C-4), 104.0 (d, C-1), 75.8 (d, C-2), 75.7 (d, C-3), 71.7 (d, C-4), 78.0 (d, C-5), 64.3 (t, C-6), 168.8 (s, C-1), 114.7 (d, C-2), 146.5 (d, C-3), 127.0 (s, C-4), 131.2 (d, C-5 , 9), 116.8 (d, C-6 , 8), 161.5 (s, C-7). These data are consistent with literature values (Tsukamoto et al., 2004), identifying compound **11** as kaempferol-3- -D-(6-O-cis-p-coumaroyl)glucopyranoside.

Compound 12 (C H O) was obtained as a white powder. ^{1}H-NMR (400 MHz, CD Cl) : 4.18 (1H, dd, J = 11.6, 4.8 Hz, H-1a), 4.13 (1H, dd, J = 11.6, 6.1 Hz, H-1b), 3.92 (1H, m, H-2), 3.69 (1H, dd, J = 11.3, 3.9 Hz, H-3a), 3.58 (1H, dd, J = 11.5, 5.9 Hz, H-3b), 2.34 (2H, t, J = 7.6 Hz, H-2), 1.61 (2H, m, H-3), 1.33-1.24 (24H, brs, H-4 to 15), 0.87 (3H, t, J = 6.8 Hz, H-16). ^{13}C-NMR (100 MHz, CD Cl) : 65.2 (t, C-1), 74.0 (d, C-2), 63.5 (t, C-3), 174.5 (s, C-1), 34.2 (t, C-2), 25.0 (t, C-3), 29.3-29.8 (t, C-4 to C-13), 32.0 (t, C-14), 22.8 (t, C-15), 14.3 (q, C-16). These data are consistent with literature values (Fu et al., 1997; Wu et al., 2005), identifying compound **12** as glyceroylmonopalmitate.

Discussion

This study isolated and identified twelve chemical constituents from the ethyl acetate fraction of the ethanol extract of Pharbitis purpurea whole herb, including four triterpenoids, four steroids, two phenylpropanoids, one flavonoid, and one fatty acid compound. Seven compounds (2–5 and 10–12) were reported from the genus Pharbitis for the first time. Literature review revealed that several of these compounds exhibit varying degrees of analgesic activity. Da et al. (2011) demonstrated that compounds 3 and 4 produce persistent analgesic and anti-inflammatory effects in persistent pain models by activating cannabi-

noid receptors, inhibiting cytokine production, and suppressing NF- B, CREB, and cyclooxygenase-2 expression, suggesting their potential as analgesic agents. Wu et al. (2011) found that compound 9 significantly inhibited xylene-induced ear swelling in mice. Our previous research also showed that compound 7 reduced writhing frequency in the acetic acid-induced mouse writhing test, indicating analgesic activity (Shan, 2017). These findings provide preliminary support for the analgesic activity of P. purpurea observed in our earlier studies. However, due to the limited quantities of isolated compounds and the lack of quantitative analysis of their overall content in the sample, it remains unclear whether these compounds are the primary analgesic principles in P. purpurea. Further investigation is needed to identify the active constituents and elucidate the underlying analgesic mechanisms.

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